UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/782,646	02/18/2004	Jens J. Hyldig-Nielsen	ABIS-001	5487

24353 7590 03/04/2008

BOZICEVIC, FIELD & FRANCIS LLP
1900 UNIVERSITY AVENUE
SUITE 200
EAST PALO ALTO, CA 94303

EXAMINER	
SALMON, KATHERINE D	
ART UNIT	PAPER NUMBER
1634	

MAIL DATE	DELIVERY MODE
03/04/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/782,646	HYLDIG-NIELSEN ET AL.	
	Examiner	Art Unit	
	KATHERINE SALMON	1634	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *15 November 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-49 and 52-56* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-49, 52-56* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 11/15/2007 has been entered.

2. Claims 1-49 and 52-56 are pending. Claims 50-51 are cancelled.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. Claims 1-49 and 52-56 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection is newly applied.

Claims 1-49 and 52-56 are indefinite over the phrase “turning-off” in claim 1 and “turning-on” in claim 1 and 43-49. It is unclear if the signal is actually turned on or off, but rather the signal is detected or not detected. It is further noted that the claims do not recite a step of turning on or turning off signal.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

4. Claims 1-4, 6, 9-10, 26-28, 31-33, 36-44, and 46-49 are rejected under 35 U.S.C. 102(b) as being anticipated by Wittwer et al. (US Patent 6,140,054 October 31, 2000). This rejection is maintained for reasons of record set forth in the office action of 12/04/2007 and as further discussed below

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes.

With regard to Claims 1, 10, 36, 46-49, Wittwer et al teaches contacting a sample with a first signal probe (donor) which is capable of hybridizing to at least a portion of a first target, a first quencher (acceptor) capable of hybridizing in proximity to the first signal probe) wherein the quencher has a Tm below that of the first signal probe (Column 4 lines 26-35, Column 12 lines 18-45). Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site

(Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (e.g. a second quencher and signal) (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the mutation then it has a lower melting temperature than the donor probe.

Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39). In Figure 1, Wittwer et al. teaches detection of fluoresce (e.g. turning on of the sample or turning off of the sample) over time as a function of melting temperature. In Figure 1, Wittwer et al. discloses rather the signal is emitted or not emitted (e.g. turned on or off). Therefore Wittwer et al. teaches monitoring the turning-off or turning-on of the signal depending on the melting temperature of the probes.

With regard to Claim 2, Wittwer teaches a method of using and monitoring fluorescent probes (Column 7 lines 60-61).

With regard to Claim 3, Wittwer et al. teaches that multiple sets of FRET pairs can be labeled with different fluorescent resonance energy transfer pairs so that the

sets of FRET oligonucleotide pairs can be distinguished from one another based on the distinguishable emission spectra (Column 12, lines 57-61).

With regard to Claim 4, Wittwer et al. teaches FRET oligonucleotide pairs having different melting temperatures for each of the FRET oligonucleotide pair is preferred (Column 13, lines 9-11).

With regard to Claims 6 and 9, Wittwer et al. teaches different sets of FRET oligonucleotide pairs can be labeled with the same fluorescent resonance transfer pair, allowing for monitoring at a single emission wavelength (Column 12 lines 49-53).

With regard to Claims 26-28 and 31-33, Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). Therefore it is inherent in the teaching of Wittwer et al. the melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

With regard to Claim 37 and 38, Wittwer et al. teaches a method in which the polynucleotide sample is an amplified product which is single stranded (Column 4, lines 20-25).

With regard to Claims 39-41, Wittwer et al. teaches multiple loci of a target nucleic acid sequence can be analyzed (column 12 lines 41-42).

With regard to Claim 42 and 44, Wittwer et al. teaches co-amplifying two or more separate regions of nucleic acid using at least two sets of PCR primers and at least two sets of FRET oligonucleotide pairs as probes to simultaneously genotype the separate regions by analyzing the melting temperature of the sets of FRET oligonucleotide pairs (Column 16 lines 11-20).

With regard to Claim 43, Wittwer et al. teaches the melting peaks of each set of probes must be distinguishable from the next set of probes (Column 15 lines 5-10). Wittwer et al. teaches to distinguish the melting point peaks of two sets of probes the probes are designed so the melting temperature of each set of probes is different from the melting temperature of the other set of probes (Column 15, lines 5-10). Wittwer et al. teaches that to differentiate amplification products the melting temperature can be separated by less than 2°C (column 16, lines 5-10).

Response to Arguments

The response traverses the rejection. A summary of the arguments is set forth below with response to arguments.

The reply asserts that the claimed invention monitors the signals from the signal flurophore (e.g. the donor dye) as opposed to conventional monitoring of the signal from the acceptor dye (quencher) (p. 13 2nd paragraph). The reply asserts that that the quencher probe is measured as a function of melting temperature (p. 13 last paragraph and p. 14 1st paragraph). The reply asserts that Wittwer monitors the acceptor whereas the present claims monitor the signals for a donor (p. 14 2nd paragraph). The reply asserts that because of this monitoring a distinct profile distinct of Wittwer et al. is made that shows the melting temperature as a function of quencher detection (p. 15 1st paragraph). The reply asserts that because of this Wittwer et al. does not teach the encompassed claims (p. 15 last paragraph).

This has been fully reviewed but is not found persuasive.

The reply seems to be asserting that there is a limiting step in the claim which measures the signal of the signal probe (e.g. the donor). However, this limitation is not found in the presently amended claims. The claims are drawn to "monitoring the detectable signals of the signal probe as a function of temperature, which comprises monitoring the turning-off of the signal of the first signal probe when the temperature is below the Tm of the first quencher probe and monitoring the turning-on of the signal of the fist signal probe when the temperature is above the Tm of the first quencher probe". The term "monitoring" is not specifically defined by the instant specification. Therefore the term can be broadly interpreted. Here in the case, Wittwer et al. teaches "to determine the melting curve for each set of donor oligonucleotide probe and acceptor oligonucleotide probes, the biological sample is illuminated with light that is absorbed by

the donor flurophore of the first and second donor oligonucleotide probes and the florescence of the sample is monitored as a function of temperature. More particularly, the fluorescence of the first and second acceptor flurophore is measured as the temperature of the sample is raised" (Column 14 lines 35-40). Therefore Wittwer et al. teaches monitoring donor oligonucleotide probes by the detection of the acceptor probe and teach all the limitations of the pending claim. However, in order to have compact prosecution a 35 USC 103(a) of Wittwer et al. in view of Tyagi et al. (SECTION 16 of the instant office action) is presented below. This 35 USC 103(a) is based on the limitation of detecting and measuring the signal of the first signal probe (detection of the donor).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

5. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein

were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

6. Claims 11-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tsourkas et al. (Nucleic acid research 2002 Vol. 30 p. 5168). This rejection is maintained for reasons of record set forth in the office action of 12/04/2007 and as further discussed below.

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et

al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39). Wittwer et al., however, does not teach the use of self-indicating signal probes such as hairpin probes.

Tsourkas et al. teaches a method of using methyl labeled molecular beacons to increase hybridization results (Abstract). With regard to Claims 11 and 12, Tsourkas teaches molecular beacons is a stem-loop confirmation (hairpin) that is quenched until it opens when hybridized to a target (2nd column 1st paragraph). With regard to Claims 13-15, Tsourkas et al. teaches that false-positive signals are fluorescence signals induced by the opening of molecular beacons due to nucleases (p. 5168 2nd column 2nd paragraph). Tsourkas et al. teaches that nuclease degradation can be reduced by adding phosphorothioate, PNA, and 2'-O-methyl (p. 5168 2nd column 2nd paragraph). Tsourkas et al. teaches that false-positives can be further reduced by using two molecule beacons that bind to adjacent regions on a target molecule and generate positive signals via FRET (p. 5168 2nd column 2nd paragraph).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use hairpin probes as signal probes as taught by Tsourkas et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Tsourkas et al. teaches

tem-loop structure of a molecular beacon improves the specificity of target discrimination compared with linear probes (p. 5168 2nd column 1st paragraph). Tsourkas et al. teaches the competing reaction between hairpin formation and target hybridization increases the sensitivity of detecting a SNP between probe and target sequences and thus enables molecular beacons to differentiated between wild-type and mutants targets better than linear probes (p. 5168 2nd column 1st paragraph).

Response to Arguments

The response traverses the rejection. The response asserts Wittwer et al. basis of detection is on the signal of the acceptor flurophore whereas Claim 1 is drawn to detection of the signal from the signal molecule (p. 14 1st paragraph). This argument has been thoroughly reviewed but is not found persuasive. This argument has not been found persuasive as discussed in the Response to Arguments in the Wittwer et al. 102(b) rejection. Specifically, The term "monitoring" is not specifically defined by the instant specification. Therefore the term can be broadly interpreted. Here in the case, Wittwer et al. teaches "to determine the melting curve for each set of donor oligonucleotide probe and acceptor oligonucleotide probes, the biological sample is illuminated with light that is absorbed by the donor flurophore of the first and second donor oligonucleotide probes and the fluorescence of the sample is monitored as a function of temperature. More particularly, the fluorescence of the first and second acceptor flurophore is measured as the temperature of the sample is raised" (Column 14 lines 35-40). Therefore Wittwer et al. teaches monitoring donor oligonucleotide probes by the detection of the acceptor probe and teach all the limitations of the

pending claim. However, in order to have compact prosecution a 35 USC 103(a) of Wittwer et al. in view of is presented below.

7. Claims 11-15 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Sokol et al. (PNAS 1998 Vol 95 p. 11538). This rejection is maintained for reasons of record set forth in the office action of 12/04/2007 and as further discussed below.

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the

mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al., however, does not teach the use of self-indicating signal probes such as hairpin probes.

Sokol et al. teaches a method of real time detection of DNA and RNA hybridization in living cells (Abstract). With regard to Claims 12-13, Sokol et al. teaches the use of Molecular beacons (hairpin probes) as the reporter (signal) oligonucleotide (abstract). The instant specification defines "self-indicating probes" as a signal probe that produces little or no detectable signal when free in solution (p. 7-8 paragraph 80). Sokol et al. teaches molecular beacons when in the stem loop confirmation (not hybridized so therefore in solution) produce no signal emission (abstract). With regard to Claim 13, Sokol et al. teaches molecular beacons provide greater nuclease resistance (p. 11541 last sentence). With regard to Claims 14-15, Sokol et al. teaches using DNA molecular beacons (p. 11538 last paragraph).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use molecular beacons as signal probes as taught by Sokol et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Sokol et al. teaches a potential problem with FRET detection is that the loss of fluorescence might not mean conclusively that hybridization has taken place therefore the use of molecular beacons

allows for direct demonstration that duplex formation took place and FRET was suppressed (p. 11538 2nd column first full paragraph).

Response to Arguments

The response traverses the rejection. The response asserts Wittwer et al. basis of detection is on the signal of the acceptor flurophore whereas Claim 1 is drawn to detection of the signal from the signal molecule (p. 14 1st paragraph). This argument has been thoroughly reviewed but is not found persuasive. This argument has not been found persuasive as discussed in the Response to Arguments in the Wittwer et al. 102(b) rejection. Specifically, The term "monitoring" is not specifically defined by the instant specification. Therefore the term can be broadly interpreted. Here in the case, Wittwer et al. teaches "to determine the melting curve for each set of donor oligonucleotide probe and acceptor oligonucleotide probes, the biological sample is illuminated with light that is absorbed by the donor flurophore of the first and second donor oligonucleotide probes and the fluorescence of the sample is monitored as a function of temperature. More particularly, the fluorescence of the first and second acceptor flurophore is measured as the temperature of the sample is raised" (Column 14 lines 35-40). Therefore Wittwer et al. teaches monitoring donor oligonucleotide probes by the detection of the acceptor probe and teach all the limitations of the pending claim. However, in order to have compact prosecution a 35 USC 103(a) of Wittwer et al. in view of is presented below.

8. Claims 11, 16-20, and 38 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Kubista et al (US Patent 6329144 December 11, 2001). This rejection is maintained for reasons of record set forth in the office action of 12/04/2007 and as further discussed below.

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al.

teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al., however, does not teach the use of self-indicating signal probes.

Kubista et al. teaches a method of using a probe for the detecting nucleic acids having a particular sequence (Abstract). With regard to Claims 11, 16, and 18-20, Kubista et al. teaches the use of PNA based probes. The instant specification defines "self-indicating probes" as a signal probe that produces little or no detectable signal when free in solution (p. 7-8 paragraph 80). Kubista et al. teaches a method that has minimal luminescence free in solution and strong luminescence bound to nucleic acids (Column 6 lines 43-45). With regard to Claim 17, Kubista et al. teaches that PNA based probes are resistant to nucleases (Column 9, line 6). With regard to Claim 38, Kubista et al. teaches the PNA forms a more rigid duplex with single stranded than double stranded nucleic acids which more effectively restricts the internal motion in the bound reporter and increases fluorescence signal (Column 10 lines 63-65).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use PNA probes as taught by Kubista et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Kubista et al. teaches PNA based probes obtain an higher signal than nucleic acid based probes when hybridized to the target strand (Column 18, lines 56-57). The ordinary artisan would use a method, which had the highest measurable signal in order to be able to determine signal versus background signal more efficiently.

Response to Arguments

The response traverses the rejection. The response asserts Wittwer et al. basis of detection is on the signal of the acceptor flurophore whereas Claim 1 is drawn to detection of the signal from the signal molecule (p. 14 1st paragraph). This argument has been thoroughly reviewed but is not found persuasive. This argument has not been found persuasive as discussed in the Response to Arguments in the Wittwer et al. 102(b) rejection. Specifically, The term "monitoring" is not specifically defined by the instant specification. Therefore the term can be broadly interpreted. Here in the case, Wittwer et al. teaches "to determine the melting curve for each set of donor oligonucleotide probe and acceptor oligonucleotide probes, the biological sample is illuminated with light that is absorbed by the donor flurophore of the first and second donor oligonucleotide probes and the fluorescence of the sample is monitored as a function of temperature. More particularly, the fluorescence of the first and second acceptor flurophore is measured as the temperature of the sample is raised" (Column 14 lines 35-40). Therefore Wittwer et al. teaches monitoring donor oligonucleotide probes by the detection of the acceptor probe and teach all the limitations of the pending claim. However, in order to have compact prosecution a 35 USC 103(a) of Wittwer et al. in view of is presented below.

9. Claims 11 and 21-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tyagi et al (US

Patent 6277607 August 21, 2001). This rejection is maintained for reasons of record set forth in the office action of 12/04/2007 and as further discussed below.

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al., however, does not teach using an intercalating dye.

Tyagi et al. teaches a method of nucleic acid amplification in which primers are in hairpin structures (abstract). With regard to Claim 11, Tyagi et al. teaches the use of hairpin primers, which can monitor the amplification reactions by florescence (Column 6, lines 52-53). With regard to Claims 21-23, Tyagi et al. teaches that the assays can be detected using intercalating dyes (Column 4, lines 6-10). Tyagi et al. teaches that one type of intercalating dye that can be used is SYBR Green (Column 11, lines 39-41).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use hairpin probes and intercalating dye as taught by Tyagi et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Tyagi et al. teaches the use of hairpin probes reduces the probability of primer dimers, allows for real-time detection of the amplification product for accurate quantification of the initial number of target sequences in a sample (Column 7, lines 1-15). Tyagi et al. teaches that the use of an intercalating dye allows for the monitoring of each reaction (Column 11, lines 35-40).

Response to Arguments

The response traverses the rejection. The response asserts Wittwer et al. basis of detection is on the signal of the acceptor flurophore whereas Claim 1 is drawn to detection of the signal from the signal molecule (p. 14 1st paragraph). This argument has been thoroughly reviewed but is not found persuasive. This argument has not been found persuasive as discussed in the Response to Arguments in the Wittwer et al. 102(b) rejection. Specifically, The term “monitoring” is not specifically defined by the

instant specification. Therefore the term can be broadly interpreted. Here in the case, Wittwer et al. teaches "to determine the melting curve for each set of donor oligonucleotide probe and acceptor oligonucleotide probes, the biological sample is illuminated with light that is absorbed by the donor flurophore of the first and second donor oligonucleotide probes and the florescence of the sample is monitored as a function of temperature. More particularly, the fluorescence of the first and second acceptor flurophore is measured as the temperature of the sample is raised" (Column 14 lines 35-40). Therefore Wittwer et al. teaches monitoring donor oligonucleotide probes by the detection of the acceptor probe and teach all the limitations of the pending claim. However, in order to have compact prosecution a 35 USC 103(a) of Wittwer et al. in view of is presented below.

10. Claims 24-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Singer et al. (US Patent 6,323,337 November 27, 2001). This rejection is maintained for reasons of record set forth in the office action of 12/04/2007 and as further discussed below.

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site

(Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al., however, does not teach the use a fluorescent minor groove binding dye.

Singer et al. teaches a method to label oligonucleotides (abstract). With regard to Claims 24 and 25, Singer et al. teaches the use of nucleic acid stain such as Hoechst 33342, Hoechst 34580, and DAPI (Column 14 lines 19-25).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use intercalating dye as taught by Singer et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Singer et al. teaches dyes, which are useful in the combination of quenching oligonucleotides to minimize the fluorescent signal from selected oligonucleotides (Column 16, lines19-22).

Response to Arguments

The response traverses the rejection. The response asserts Wittwer et al. basis of detection is on the signal of the acceptor flurophore whereas Claim 1 is drawn to detection of the signal from the signal molecule (p. 14 1st paragraph). This argument has been thoroughly reviewed but is not found persuasive. This argument has not been found persuasive as discussed in the Response to Arguments in the Wittwer et al. 102(b) rejection. Specifically, The term "monitoring" is not specifically defined by the instant specification. Therefore the term can be broadly interpreted. Here in the case, Wittwer et al. teaches "to determine the melting curve for each set of donor oligonucleotide probe and acceptor oligonucleotide probes, the biological sample is illuminated with light that is absorbed by the donor flurophore of the first and second donor oligonucleotide probes and the fluorescence of the sample is monitored as a function of temperature. More particularly, the fluorescence of the first and second acceptor flurophore is measured as the temperature of the sample is raised" (Column 14 lines 35-40). Therefore Wittwer et al. teaches monitoring donor oligonucleotide probes by the detection of the acceptor probe and teach all the limitations of the pending claim. However, in order to have compact prosecution a 35 USC 103(a) of Wittwer et al. in view of is presented below.

11. Claim 45 is rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Schalasta et al. (Infection 2000

Vol 28 p85). This rejection is maintained for reasons of record set forth in the office action of 12/04/2007 and as further discussed below.

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al., however, does not teach using the method for virus genotyping.

With regard to Claim 45, Schalasta et al. teaches a method of genotyping type-specific HPV Type 1 and Type 2 using fluorescence Melting Curve analysis (Abstract).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use in genotyping viruses as taught by Schalasta et al. The ordinary artisan would have been motivated to use the method of Wittwer et al. because Schalasta et al. teaches the use of FRET based melting curve analysis provides a rapid diagnosis, high sensitivity, and specificity (abstract).

Response to Arguments

The response traverses the rejection. The response asserts Wittwer et al. basis of detection is on the signal of the acceptor flurophore whereas Claim 1 is drawn to detection of the signal from the signal molecule (p. 14 1st paragraph). This argument has been thoroughly reviewed but is not found persuasive. This argument has not been found persuasive as discussed in the Response to Arguments in the Wittwer et al. 102(b) rejection. Specifically, The term "monitoring" is not specifically defined by the instant specification. Therefore the term can be broadly interpreted. Here in the case, Wittwer et al. teaches "to determine the melting curve for each set of donor oligonucleotide probe and acceptor oligonucleotide probes, the biological sample is illuminated with light that is absorbed by the donor flurophore of the first and second donor oligonucleotide probes and the fluorescence of the sample is monitored as a function of temperature. More particularly, the fluorescence of the first and second

acceptor flurophore is measured as the temperature of the sample is raised" (Column 14 lines 35-40). Therefore Wittwer et al. teaches monitoring donor oligonucleotide probes by the detection of the acceptor probe and teach all the limitations of the pending claim. However, in order to have compact prosecution a 35 USC 103(a) of Wittwer et al. in view of is presented below.

12. Claims 5 and 7-8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Caplin et al. (Biochemical 1999 No. 1 p. 5). This rejection is maintained for reasons of record set forth in the office action of 12/04/2007 and as further discussed below.

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et

al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

With regard to Claim 8, Wittwer et al. teaches to distinguish the melting point peaks of two sets of probes the probes are designed so the melting temperature of each set of probes is different from the melting temperature of the other set of probes (Column 15, lines 5-10). Wittwer et al. teaches that to differentiate amplification products the melting temperature can be separated by less than 2°C (column 16, lines 5-10).

Wittwer et al., however, does not teach the melting temperature differences between the probes.

Caplin et al. teaches a method of direct mutation detection (abstract). With regard to Claims 5 and 7, Caplin et al. teaches for mutation detection, the best melting curves are obtained when the difference between the probes (quencher and signal) is between 5-10°C (p. 6 1st paragraph). With regard to Claim 8, Caplin teaches that for detection the melting temperature of hybridization probes should be within 2°C (p. 6 1st paragraph).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use the

melting temperature differences between probes as taught by Caplin et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Caplin et al. teaches the best melting curves are obtained with the specific temperature difference of 5-10°C (p. 6 1st paragraph). The ordinary artisan would be motivated to create the second signal probe at least 7 degrees less than the first signal probe to ensure that the second set could be detected (5 degrees from the quencher and 2 degrees below the other hybridized probe).

Response to Arguments

The response traverses the rejection. The response asserts Wittwer et al. basis of detection is on the signal of the acceptor flurophore whereas Claim 1 is drawn to detection of the signal from the signal molecule (p. 14 1st paragraph). This argument has been thoroughly reviewed but is not found persuasive. This argument has not been found persuasive as discussed in the Response to Arguments in the Wittwer et al. 102(b) rejection. Specifically, The term "monitoring" is not specifically defined by the instant specification. Therefore the term can be broadly interpreted. Here in the case, Wittwer et al. teaches "to determine the melting curve for each set of donor oligonucleotide probe and acceptor oligonucleotide probes, the biological sample is illuminated with light that is absorbed by the donor flurophore of the first and second donor oligonucleotide probes and the fluorescence of the sample is monitored as a function of temperature. More particularly, the fluorescence of the first and second acceptor flurophore is measured as the temperature of the sample is raised" (Column 14 lines 35-40). Therefore Wittwer et al. teaches monitoring donor oligonucleotide

probes by the detection of the acceptor probe and teach all the limitations of the pending claim. However, in order to have compact prosecution a 35 USC 103(a) of Wittwer et al. in view of is presented below.

13. Claims 29 and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Marras et al. (Nucleic Acids Research 2002 Vol 30 p. e122). This rejection is maintained for reasons of record set forth in the office action of 12/04/2007 and as further discussed below.

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the

mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). The melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

Wittwer et al., however, does not teach the temperature rate range.

Marras et al. teaches a method of real-time fluorescence using a rapid cycle PCR. Marras et al. teaches that using the 7700 Prism spectroflurometric thermal cycler the temperature was raised in increments of 1°C/minute.

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use the ramping speed as taught by Marras et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Marras et al. teaches the ramping speed acceptable to a commercially produced thermal cycler. The ordinary

artisan would want to use the ramping speed that gives the best results for detection of melting temperature.

Response to Arguments

The response traverses the rejection. The response asserts Wittwer et al. basis of detection is on the signal of the acceptor flurophore whereas Claim 1 is drawn to detection of the signal from the signal molecule (p. 14 1st paragraph). This argument has been thoroughly reviewed but is not found persuasive. This argument has not been found persuasive as discussed in the Response to Arguments in the Wittwer et al. 102(b) rejection. Specifically, The term "monitoring" is not specifically defined by the instant specification. Therefore the term can be broadly interpreted. Here in the case, Wittwer et al. teaches "to determine the melting curve for each set of donor oligonucleotide probe and acceptor oligonucleotide probes, the biological sample is illuminated with light that is absorbed by the donor flurophore of the first and second donor oligonucleotide probes and the fluorescence of the sample is monitored as a function of temperature. More particularly, the fluorescence of the first and second acceptor flurophore is measured as the temperature of the sample is raised" (Column 14 lines 35-40). Therefore Wittwer et al. teaches monitoring donor oligonucleotide probes by the detection of the acceptor probe and teach all the limitations of the pending claim. However, in order to have compact prosecution a 35 USC 103(a) of Wittwer et al. in view of is presented below.

14. Claims 29 and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Elenitoba-Johnson (US Patent 6346386 February 12, 2002). This rejection is maintained for reasons of record set forth in the office action of 12/04/2007 and as further discussed below.

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al.

teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). The melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

Wittwer et al., however, does not teach the temperature rate range.

Elenitoba-Johnson teaches a method for determining alterations in a nucleic acid using a fluorescent label and melting profiles (Abstract). With regard to Claims 29 and 34, Elenitoba-Johnson teaches a method of performing RT PCR in which the temperature of the sample is raised at a rate slow enough to distinguish between the melting points of the wild type and the fragment of interest (Column 7 lines 5-10). Elenitoba-Johnson teaches a ramping rate of between 0.1 °C/sec to 0.01°C/sec (6°C/min to .6 °C/min) (Column 7, lines 11-20). Elenitoba-Johnson teaches a PCR amplification method in which the probes and target are increased above the melting point of the probes to below the melting point of the probes (Column 10 Example 3).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use the ramping speed as taught by Elenitoba-Johnson et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Elenitoba-Johnson et al. teaches a ramping speed in which it is slow enough to distinguish between the melting temperatures of two sequences (Column 7, lines 1-5).

Response to Arguments

The response traverses the rejection. The response asserts Wittwer et al. basis of detection is on the signal of the acceptor flurophore whereas Claim 1 is drawn to detection of the signal from the signal molecule (p. 14 1st paragraph). This argument has been thoroughly reviewed but is not found persuasive. This argument has not been found persuasive as discussed in the Response to Arguments in the Wittwer et al. 102(b) rejection. Specifically, The term "monitoring" is not specifically defined by the instant specification. Therefore the term can be broadly interpreted. Here in the case, Wittwer et al. teaches "to determine the melting curve for each set of donor oligonucleotide probe and acceptor oligonucleotide probes, the biological sample is illuminated with light that is absorbed by the donor flurophore of the first and second donor oligonucleotide probes and the fluorescence of the sample is monitored as a function of temperature. More particularly, the fluorescence of the first and second acceptor flurophore is measured as the temperature of the sample is raised" (Column 14 lines 35-40). Therefore Wittwer et al. teaches monitoring donor oligonucleotide probes by the detection of the acceptor probe and teach all the limitations of the

pending claim. However, in order to have compact prosecution a 35 USC 103(a) of Wittwer et al. in view of is presented below.

15. Claims 30 and 35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Witter et al. (named Witter B) (US Patent 6,245,514 June 2001) . This rejection is maintained for reasons of record set forth in the office action of 12/04/2007 and as further discussed below.

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes. Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (Column 12, lines 40-45). Wittwer et al. teaches that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-

34). Wittwer teaches that when the acceptor probe is designed to hybridize to the mutation then it has a lower melting temperature than the donor probe. Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39).

Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). The melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

Wittwer et al., however, does not teach the rate of monitoring the detectable signal.

Wittwer B teaches a method of measuring FRET pairs for detecting the presences of a target analyte (Abstract). Wittwer B teaches measuring fluorescent continuously every 200 msec as a function of temperature (Column 35 lines 29-33).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. to use the monitoring rate as taught by Wittwer B. The ordinary artisan would have been motivated to modify the method of Wittwer et al. because Wittwer B teaches a monitoring rate in

which data can be obtained by measuring fluorescence at each temperature (Column 37, lines 1-5). The ordinary artisan would want to modify the method in order to gain as much information as possible during the monitoring phase in order to make a more precise fluorescence vs. melting temperature curve.

Response to Arguments

The response traverses the rejection. The response asserts Wittwer et al. basis of detection is on the signal of the acceptor flurophore whereas Claim 1 is drawn to detection of the signal from the signal molecule (p. 14 1st paragraph). This argument has been thoroughly reviewed but is not found persuasive. This argument has not been found persuasive as discussed in the Response to Arguments in the Wittwer et al. 102(b) rejection. Specifically, The term "monitoring" is not specifically defined by the instant specification. Therefore the term can be broadly interpreted. Here in the case, Wittwer et al. teaches "to determine the melting curve for each set of donor oligonucleotide probe and acceptor oligonucleotide probes, the biological sample is illuminated with light that is absorbed by the donor flurophore of the first and second donor oligonucleotide probes and the fluorescence of the sample is monitored as a function of temperature. More particularly, the fluorescence of the first and second acceptor flurophore is measured as the temperature of the sample is raised" (Column 14 lines 35-40). Therefore Wittwer et al. teaches monitoring donor oligonucleotide probes by the detection of the acceptor probe and teach all the limitations of the pending claim. However, in order to have compact prosecution a 35 USC 103(a) of Wittwer et al. in view of is presented below.

16. Claims 1-4, 6, 9-12, 14-15, 26-28, 31-33, 36-44, 46-56 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tyagi et al. (Nature Biotechnology January 1998 VOl. 16 p. 49) as evidenced by Didenko et al. (Biotechniques 2001 VOl 31 p. 1106). This rejection is newly applied.

Wittwer et al. teaches a method of mutation detection using fluorescently labeled oligonucleotide hybridization probes to identify mutations and polymorphisms based on melting curve analysis of hybridization probes.

With regard to Claims 1, 10, 36, 46-49, Wittwer et al teaches contacting a sample with a first signal probe (donor) which is capable of hybridizing to at least a portion of a first target, a first quencher (acceptor) capable of hybridizing in proximity to the first signal probe) wherein the quencher has a Tm below that of the first signal probe (Column 4 lines 26-35, Column 12 lines 18-45). Wittwer et al. teaches the detection of sequence alterations at two or more loci of an amplified DNA sequence determined by melting-point analysis (Column 4 lines 20-25). Wittwer et al. teaches hybridization probes are designed to hybridize to the amplified region and span the mutation site (Column 4, lines 26-29). Wittwer et al. teaches a method using FRET oligonucleotide pair comprised of a donor and acceptor (Column 4 lines 32-35). Wittwer et al teaches a method in which multiple loci of the target nucleic acids sequence can be analyzed using sets of FRET oligonucleotide pairs in which have different melting temperatures (e.g. a second quencher and signal) (Column 12, lines 40-45). Wittwer et al. teaches

that either the donor or acceptor (signal or quencher) can pan across the mutation (Column 12, lines 18-20). Wittwer et al. teaches the melting temperature of the probe hybridized to the mutation locus has a lower melting temperature then the other probe in the FRET pair (column 12, lines 29-34). Wittwer teaches that when the acceptor probe is designed to hybridize to the mutation then it has a lower melting temperature than the donor probe.

Wittwer et al. teaches the fluorescence of the sample is monitored as a function of temperature (Column 14, lines 38-39). In Figure 1, Wittwer et al. teaches detection of fluoresce (e.g. turning on of the sample or turning off of the sample) over time as a function of melting temperature. In Figure 1, Wittwer et al. discloses rather the signal is emitted or not emitted (e.g. turned on or off). Therefore Wittwer et al. teaches monitoring the turning-off or turning-on of the signal depending on the melting temperature of the probes.

With regard to Claim 2, Wittwer teaches a method of using and monitoring fluorescent probes (Column 7 lines 60-61).

With regard to Claim 3, Wittwer et al. teaches that multiple sets of FRET pairs can be labeled with different fluorescent resonance energy transfer pairs so that the sets of FRET oligonucleotide pairs can be distinguished from one another based on the distinguishable emission spectra (Column 12, lines 57-61).

With regard to Claim 4, Wittwer et al. teaches FRET oligonucleotide pairs having different melting temperatures for each of the FRET oligonucleotide pair is preferred (Column 13, lines 9-11).

With regard to Claims 6 and 9, Wittwer et al. teaches different sets of FRET oligonucleotide pairs can be labeled with the same fluorescent resonance transfer pair, allowing for monitoring at a single emission wavelength (Column 12 lines 49-53).

With regard to Claims 26-28 and 31-33, Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). Therefore it is inherent in the teaching of Wittwer et al. the melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

With regard to Claim 37, Wittwer et al. teaches a method in which the polynucleotide sample is an amplified product (Column 4, lines 20-25).

With regard to Claims 39-41, Wittwer et al. teaches multiple loci of a target nucleic acid sequence can be analyzed (column 12 lines 41-42).

With regard to Claim 42 and 44, Wittwer et al. teaches co-amplifying two or more separate regions of nucleic acid using at least two sets of PCR primers and at least two sets of FRET oligonucleotide pairs as probes to simultaneously genotype the separate

regions by analyzing the melting temperature of the sets of FRET oligonucleotide pairs (Column 16 lines 11-20).

With regard to Claim 43, Wittwer et al. teaches the melting peaks of each set of probes must be distinguishable from the next set of probes (Column 15 lines 5-10). Wittwer et al. teaches to distinguish the melting point peaks of two sets of probes the probes are designed so the melting temperature of each set of probes is different from the melting temperature of the other set of probes (Column 15, lines 5-10). Wittwer et al. teaches that to differentiate amplification products the melting temperature can be separated by less than 2°C (column 16, lines 5-10).

However, Wittwer et al. does not teach a method in which the signal probe is monitored by the direct detection of the signal probe.

Tyagi et al. teaches a method of detection of allele discrimination using a quencher and a signal (abstract). With regard to Claims 1, 10, 36, 46-49, Tyagi et al. teaches that to measure of the fluorescence of the solution to determine the extent of quenching the flurophore (e.g. the signal moiety) is measured (p. 49 last paragraph). Therefore, Tyagi et al. teaches detection of the turning off and turning on of the signal by detection of the fluorescence of the signal.

The instant specification defines “self-indicating probes” as a signal probe that produces little or no detectable signal when free in solution (p. 7-8 paragraph 80). With regard to Claims 11-12, Tyagi et al. teaches molecular beacons when in the stem loop confirmation (not hybridized so therefore in solution) produce no signal emission (abstract).

With regard to Claims 14 and 15, Tyagi et al. teaches the probes are DNA (p. 53 1st column last paragraph).

With regard to Claims 52-56, Tyagi et al. teaches a method wherein the quencher is non-fluorescent. (p. 49 1st column 1st paragraph). Tyagi et al. teaches that this quencher is DABCYL (p. 49 2nd column last paragraph). Didenko et al. teaches that DABCY-based quenching is not FRET related (p. 1108 3rd column 1st pa[ra]graph).

Therefore it would be prima facie obvious to one of ordinary skill in the art at the time the invention was filed to modify the method of target detection by detection fluorescence as a function of temperature as taught by Wittwer to detect the signal probe to determine if fluorescence occurred as taught by Tyagi et al. The ordinary artisan would be motivated to modify the method of target detection by detection fluorescence as a function of temperature as taught by Wittwer to detect the signal probe to determine if fluorescence occurred as taught by Tyagi et al. because Tyagi et al. teaches that by using a quencher such as DABCYL which does not fluorescence and measuring the extent of quenching by detecting the signal probe one can detect flurophores (signals) of any color simultaneously such that the fluorescence of each is distinguishable from the fluorescence of the other signal probes (p. 49 last paragraph, p. 50 1st sentence 51 1st full paragraph). Therefore the ordinary artisan would be motivated to detect the signal probe and to use a quencher with no fluorescence in order to measure multiple signals from the signal probes without interference of the quencher.

17. Claims 5 and 7-8 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tyagi et al. (Nature Biotechnology January 1998 VOl. 16 p. 49) as evidenced by Didenko et al. (Biotechniques 2001 VOl 31 p. 1106) as applied to claims 1-4, 6, 9-12, 14-15, 26-28, 31-33, 36-44, 46-56 in view of Caplin et al. (Biochemical 1999 No. 1 p. 5). This rejection is newly applied.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. teach a method of analyzing a polynucleotide sample for on or more targets by monitoring the detectable signals of the signal probes as a function of temperature.

With regard to Claim 8, Wittwer et al. teaches to distinguish the melting point peaks of two sets of probes the probes are designed so the melting temperature of each set of probes is different from the melting temperature of the other set of probes (Column 15, lines 5-10). Wittwer et al. teaches that to differentiate amplification products the melting temperature can be separated by less than 2°C (column 16, lines 5-10).

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al., however, does not teach the melting temperature differences between the probes.

Caplin et al. teaches a method of direct mutation detection (abstract). With regard to Claims 5 and 7, Caplin et al. teaches for mutation detection, the best melting curves are obtained when the difference between the probes (quencher and signal) is between 5-10°C (p. 6 1st paragraph). With regard to Claim 8, Caplin teaches that for

detection the melting temperature of hybridization probes should be within 2°C (p. 6 1st paragraph).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. to use the melting temperature differences between probes as taught by Caplin et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. because Caplin et al. teaches the best melting curves are obtained with the specific temperature difference of 5-10°C (p. 6 1st paragraph). The ordinary artisan would be motivated to create the second signal probe at least 7 degrees less than the first signal probe to ensure that the second set could be detected (5 degrees from the quencher and 2 degrees below the other hybridized probe).

18. Claims 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tyagi et al. (Nature Biotechnology January 1998 VOl. 16 p. 49) as evidenced by Didenko et al. (Biotechniques 2001 VOl 31 p. 1106) as applied to claims 1-4, 6, 9-12, 14-15, 26-28, 31-33, 36-44, 46-56 in view of Sokol et al. (PNAS 1998 Vol 95 p. 11538). This rejection is newly applied.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. teach a method of analyzing a polynucleotide sample for on or more targets by monitoring the detectable

signals of the signal probes as a function of temperature in which the probes are molecular beacons.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al., however, do not teach the use of self-indicating signal probes such as hairpin probes.

Sokol et al. teaches a method of real time detection of DNA and RNA hybridization in living cells (Abstract). With regard to Claims 13, Sokol et al. teaches the use of Molecular beacons (hairpin probes) as the reporter (signal) oligonucleotide (abstract). The instant specification defines "self-indicating probes" as a signal probe that produces little or no detectable signal when free in solution (p. 7-8 paragraph 80). Sokol et al. teaches molecular beacons when in the stem loop confirmation (not hybridized so therefore in solution) produce no signal emission (abstract). Sokol et al. teaches molecular beacons provide greater nuclease resistance (p. 11541 last sentence).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. to use molecular beacons as signal probes as taught by Sokol et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. because Sokol et al. teaches a potential problem with FRET detection is that the loss of fluorescence might not mean conclusively that hybridization has taken place therefore the use of molecular beacons allows for direct demonstration that duplex formation took place and FRET was suppressed (p. 11538 2nd column first full paragraph).

19. Claims 16-20 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tyagi et al. (Nature Biotechnology January 1998 VOl. 16 p. 49) as evidenced by Didenko et al. (Biotechniques 2001 VOl 31 p. 1106) as applied to claims 1-4, 6, 9-12, 14-15, 26-28, 31-33, 36-44, 46-56 in view of Kubista et al (US Patent 6329144 December 11, 2001). This rejection is newly applied.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. teach a method of analyzing a polynucleotide sample for on or more targets by monitoring the detectable signals of the signal probes as a function of temperature in which the probes are molecular beacons.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al., however, do not teach the use of linear self-indicating signal probes.

Kubista et al. teaches a method of using a probe for the detecting nucleic acids having a particular sequence (Abstract). With regard to Claims 16-20, Kubista et al. teaches the use of PNA based probes. The instant specification defines "self-indicating probes" as a signal probe that produces little or no detectable signal when free in solution (p. 7-8 paragraph 80). Kubista et al. teaches a method that has minimal luminescence free in solution and strong luminescence bound to nucleic acids (Column 6 lines 43-45). With regard to Claim 17, Kubista et al. teaches that PNA based probes are resistant to nucleases (Column 9, line 6).

With regard to Claim 38, Kubista et al. teaches the PNA forms a more rigid duplex with single stranded than double stranded nucleic acids which more effectively restricts

the internal motion in the bound reporter and increases fluorescence signal (Column 10 lines 63-65).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. to use PNA probes (linear self indicating probes) as taught by Kubista et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. because Kubista et al. teaches PNA based probes obtain an higher signal than nucleic acid based probes when hybridized to the target strand (Column 18, lines 56-57). The ordinary artisan would use a method, which had the highest measurable signal in order to be able to determine signal versus background signal more efficiently.

20. Claims 21-23 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tyagi et al. (Nature Biotechnology January 1998 VOl. 16 p. 49) as evidenced by Didenko et al. (Biotechniques 2001 VOl 31 p. 1106) as applied to claims 1-4, 6, 9-12, 14-15, 26-28, 31-33, 36-44, 46-56 in view of Tyagi et al (US Patent 6277607 August 21, 2001)(known as Tyagi B). This rejection is newly applied.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. teach a method of analyzing a polynucleotide sample for on or more targets by monitoring the detectable

signals of the signal probes as a function of temperature in which the probes are molecular beacons.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al., however, do not teach not teach using an intercalating dye.

Tyagi B et al. teaches a method of nucleic acid amplification in which primers are in hairpin structures (abstract). Tyagi B et al. teaches the use of hairpin primers, which can monitor the amplification reactions by florescence (Column 6, lines 52-53). With regard to Claims 21-23, Tyagi B et al. teaches that the assays can be detected using intercalating dyes (Column 4, lines 6-10). Tyagi B et al. teaches that one type of intercalating dye that can be used is SYBR Green (Column 11, lines 39-41).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. to use hairpin probes and intercalating dye as taught by Tyagi B et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. because Tyagi B et al. teaches the use of hairpin probes reduces the probability of primer dimers, allows for real-time detection of the amplification product for accurate quantification of the initial number of target sequences in a sample (Column 7, lines 1-15). Tyagi B et al. teaches that the use of an intercalating dye allows for the monitoring of each reaction (Column 11, lines 35-40).

21. Claims 24-25 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tyagi et al. (Nature Biotechnology January 1998 VOl. 16 p. 49) as evidenced by Didenko et al. (Biotechniques 2001 VOl 31 p. 1106) as applied to claims 1-4, 6, 9-12, 14-15, 26-28, 31-33, 36-44, 46-56 in view of Singer et al. (US Patent 6,323,337 November 27, 2001). This rejection is newly applied.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. teach a method of analyzing a polynucleotide sample for on or more targets by monitoring the detectable signals of the signal probes as a function of temperature in which the probes are molecular beacons.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al., however, do not teach does not teach the use a fluorescent minor groove binding dye.

Singer et al. teaches a method to label oligonucleotides (abstract). With regard to Claims 24 and 25, Singer et al. teaches the use of nucleic acid stain such as Hoechst 33342, Hoechst 34580, and DAPI (Column 14 lines 19-25).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al.. to use intercalating dye as taught by Singer et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. because Singer et al. teaches dyes, which are useful in the combination of quenching oligonucleotides to minimize the fluorescent signal from selected oligonucleotides (Column 16, lines19-22).

22. Claims 29 and 34 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tyagi et al. (Nature Biotechnology January 1998 VOl. 16 p. 49) as evidenced by Didenko et al. (Biotechniques 2001 VOl 31 p. 1106) as applied to claims 1-4, 6, 9-12, 14-15, 26-28, 31-33, 36-44, 46-56 in view of Elenitoba-Johnson (US Patent 6346386 February 12, 2002). This rejection is newly applied.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. teach a method of analyzing a polynucleotide sample for on or more targets by monitoring the detectable signals of the signal probes as a function of temperature in which the probes are molecular beacons.

Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). The melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al., however, do not not teach the temperature rate range.

Elenitoba-Johnson teaches a method for determining alterations in a nucleic acid using a fluorescent label and melting profiles (Abstract). With regard to Claims 29 and 34, Elenitoba-Johnson teaches a method of performing RT PCR in which the temperature of the sample is raised at a rate slow enough to distinguish between the melting points of the wild type and the fragment of interest (Column 7 lines 5-10). Elenitoba-Johnson teaches a ramping rate of between 0.1 °C/sec to 0.01°C/sec (6°C/min to .6 °C/min) (Column 7, lines 11-20). Elenitoba-Johnson teaches a PCR amplification method in which the probes and target are increased above the melting point of the probes to below the melting point of the probes (Column 10 Example 3).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. to use the ramping speed as taught by Elenitoba-Johnson et al. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. because Elenitoba-Johnson et al. teaches a ramping speed in which it is slow enough to distinguish between the melting temperatures of two sequences (Column 7, lines 1-5).

23. Claims 30 and 35 are rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tyagi et al. (Nature Biotechnology January 1998 VOl. 16 p. 49) as evidenced by Didenko et al. (Biotechniques 2001 VOl 31 p. 1106) as applied to claims 1-4, 6, 9-12, 14-15, 26-28,

31-33, 36-44, 46-56 in view of Witter et al. (named Witter B) (US Patent 6,245,514 June 2001). This rejection is newly applied.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. teach a method of analyzing a polynucleotide sample for on or more targets by monitoring the detectable signals of the signal probes as a function of temperature in which the probes are molecular beacons.

Wittwer et al. teaches fluorescence monitoring of the PCR reaction is used to acquire product melting curves during the PCR reaction (Column 15 lines 56-57). Wittwer et al. teaches the temperature cycles of PCR that drive amplification alternately denature the accumulating product and the fluorescently labeled hybridized probes at a high temperature, and anneal the primers and the hybridization probes to the product at a lower temperature (Column 15 lines 59-63). The melting temperature is measured by as an increase in temperature and a decrease in temperature. Wittwer et al. teaches temperature cycling of 94°C to 75°C which are above the melting point of the quencher probe and below the melting point of the signal probe (Column 20 lines 25-30).

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al., however, do not teach the rate of monitoring the detectable signal.

Wittwer B teaches a method of measuring FRET pairs for detecting the presences of a target analyte (Abstract). Wittwer B teaches measuring fluorescent continuously every 200 msec as a function of temperature (Column 35 lines 29-33).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Tyagi

et al. as evidenced by DIdenko et al. to use the monitoring rate as taught by Wittwer B. The ordinary artisan would have been motivated to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. because Wittwer B teaches a monitoring rate in which data can be obtained by measuring fluorescence at each temperature (Column 37, lines 1-5). The ordinary artisan would want to modify the method in order to gain as much information as possible during the monitoring phase in order to make a more precise fluorescence vs. melting temperature curve.

24. Claim 45 is rejected under 35 U.S.C. 103(a) as being unpatentable over Wittwer et al. (US Patent 6,140,054 October 31, 2000) in view of Tyagi et al. (Nature Biotechnology January 1998 VOl. 16 p. 49) as evidenced by Didenko et al. (Biotechniques 2001 VOl 31 p. 1106) as applied to claims 1-4, 6, 9-12, 14-15, 26-28, 31-33, 36-44, 46-56 in view of Schalasta et al. (Infection 2000 Vol 28 p85). This rejection is newly applied.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. teach a method of analyzing a polynucleotide sample for on or more targets by monitoring the detectable signals of the signal probes as a function of temperature in which the probes are molecular beacons.

Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al., however, do not teach using the method for virus genotyping.

With regard to Claim 45, Schalasta et al. teaches a method of genotyping type-specific HPV Type 1 and Type 2 using fluorescence Melting Curve analysis (Abstract).

Therefore it would have been prime facie obvious to one of ordinary skill in the art at the time the invention was made to modify the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. to use in genotyping viruses as taught by Schalasta et al. The ordinary artisan would have been motivated to use the method of Wittwer et al. and Tyagi et al. as evidenced by DIdenko et al. because Schalasta et al. teaches the use of FRET based melting curve analysis provides a rapid diagnosis, high sensitivity, and specificity (abstract).

Conclusion

25. No Claims are allowed.

26. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Katherine Salmon whose telephone number is (571) 272-3316. The examiner can normally be reached on Monday-Friday 8AM-430PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ram Shukla can be reached on (571) 272-0735. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/Katherine Salmon/
Examiner, Art Unit 1634

/Ram R. Shukla/

Supervisory Patent Examiner, Art Unit 1634